ENVIRONMENTAL DEVICES, INCORPORATED

FINAL REPORT

COINCIDENTAL CHEMICAL SIGNATURE IDENTIFIER

1985

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Atch. 2

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SUMMARY

In the Phase I feasibility research study, we evaluated and tested methods for collecting, cataloguing and identifying human effluent vapors that were characteristic and unique to the individual. A variety of temperatures, enclosures, flow rates, filters and sample traps were used in the collection of vapors from three female and two male subjects in an attempt to distinguish one spectrum from another.

Specimen collection was via; 1) a whole body clean chamber in which the subject remained for 1/2 hour to 1 1/2 hours, 2) a mini-chamber, 3) direct injection of skin oils, and 4) external vaporization of skin oils.

Analysis of specimen was via the Olfax I mass spectrometer. We had arranged to lease an Olfax IIA for our research in the Phase I project, but found it necessary to obtain an earlier model due to budget cuts prior to the award of the grant. Data required from sample collection were first printed and graphed and then sorted out to determine a means of defining spectral characteristics which best differentiate one individual from another.

It was found that all our subjects' spectra were comprised of essentially the same chemical compounds, with varying abundances. The two subjects, (1 male, 1 female), most frequently and thoroughly tested, revealed nominal mass intensity ratios for 53% of the mass numbers usually monitored in our studies differing by more than 20% from the paired spectrum.' These differences suggest that distinguishing characteristics do exist from person to person and that given the proper tools and sufficient time, an instrument can be developed to meet military and commercial security requirements.

INTRODUCTION

In our proposal, we hypothesized that an individual possesses distinguishable characteristics, inherent in his chemical makeup, that differentiates him from all other people. We based this theory, in part, on evidence that lower animals, such as seals and hound dogs, have the ability when separated and then reunited to identify their pups through smell, from all others, and that the bloodhound's olfactory senses enable him to track and locate humans, as well. The Phase I research was not concerned with how an animal's sense of smell functions, but with how existing analytical chemical instrumentation could assist in deciphering vapors emitted by humans which could result in a positive chemical identification signature.

The mass spectrometer, one of the more advanced systems for chemical analysis, was employed in our research as a means to identify, classify and isolate compounds present in human spectra. In previous studies related to human effluents[1], contraband foodstuffs[2]

INTRODUCTION (continued)

researchers had limited success using the mass spectrometer due to the background contaminants present in the environmental conditions, and also because of the ionization mode they chose. In our study we had the advantages of PBM[3] software, which included the Olfax spectrometer's ability to autorange over six decades and to digitize the signals prior to printing the spectral data. This permitted us to "see" variances and to establish ratios between our subjects' spectra.

The technical objectives of the project began with the construction of a 600 liter sample acquisition chamber and the modification and refurbishing of an Olfax I mass spectrometer. Vapor specimen were collected via sample traps from subjects while in the chamber. The traps were later attached to the mass spectrometer inlet for analysis. In other experiments a direct connection line leading from the chamber to the analyzer was devised in order to evaluate sensitivity levels. A 2.0 liter mini-chamber, fashioned to collect specimen from subjects' undergarments, was used for vapor collection both by use of sample traps and direct line analysis. We had intended to examine in vitro gas mixtures during the Phase I project in order to compare the intensities of unrecorded chemical signatures with known amounts of known compounds in an attempt to estimate detection limits and to evaluate specificity. Since the early phase of the project was not greatly dependent on this data and our budget was cut more than $27,000, these experiments were postponed for the Phase II effort.

The complexity of identifying an individual by vapors emanating from the body is quite unlike placing one's hands on an ink pad and then transferring an imprint on to paper. Yet, the results of both methods of identification may one day be a reality, with some important differences. The time required for law enforcement agencies, et al, to compare an individual set of fingerprints to the many millions on file is time consuming and costly. Further, this method of identification is impractical for many purposes, such as for access control in the work place or entry to a missile silo. The major advantage to fingerprinting is, of course, that prints accompany us throughout life.

In this last sense, a Chemical Signature Identifier seems to have similar attributes, i.e., it appears to be intrinsic to the individual. Most identification systems now available, such as the "eyedentification" or hand print can change with time. The purpose of developing a chemical identifier is to establish evidence that individual chemical signatures are not altered with time or ingestion of different foodstuffs, etc., as well as to prevent falsification of identity as is possible with other forms of identification.

Although progress on this project did not meet our initial expectations due to a variety of problems (many of which were imposed upon us by budget limitations), detailed in the main text of our report, we feel that the sensitivity and specificity obtained are sufficient to demonstrate the feasibility of our approach.

SYSTEM COMPONENTS

The Olfax mass spectrometer system, used extensively in our research, includes a computer designed in 1970 by Universal Monitor Corporation, which was one of the first microcomputers fabricated in the United States. It's design includes an Intel 8008 CPU chip, 4K RAM memory and handwired PC boards. Interfaced to this is a Texas Instruments data terminal, Model 733ASR, with cassette tape storage capabilities. As the detector, the Olfax utilizes a UTI 100C, which was manufactured by Uthe Technology Incorporated, of Sunnyvale, California. An inlet for accepting liquid sample injections or air samples, is connected to the vacuum system via a double stage silicone rubber membrane separator. The membrane separator, through which atmospheric pressure samples are introduced into the mass spectrometer vacuum system, was designed in 1970 and is manufactured and sold worldwide by Mr. Mathews, Principal Investigator of the Phase I project. The significance of membrane technology to our research is reflected in the enrichment factor of 10^6 for the typical organic compounds relative to the matrix of permanent gases in which they are carried.

For clarification, a brief discussion of membrane theory follows. The ability of membranes to extract organic vapors out of permanent gases is based on "selective permeability", i.e., depending on their physical properties, one gas in a mixture can permeate through a membrane faster than a second gas. The gas mixture on one side of the membrane is depleted of the more permeable compound. The gas mixture on the other side is thereby enriched in the more permeable component. This occurs when a gas dissolves in the membrane on its high partial pressure side of the membrane, diffuses through the membrane material by a random walk process and vaporizes again to a gas on the low partial pressure side. The transmission of various organic compounds depends upon their diffusion rates (directly temperature dependent) and their solubilities (inversely temperature dependent). Of these, solubility, which is a function of molecular weight and polarity, is by far the most important.

DISCUSSION

Since the differences in chemical signatures of various individuals is expected to be very subtle, a very sophisticated mass spectral matching technique must be employed. By far, the most advanced of such pattern matching procedures is Professor Fred W. McLafferty's Probability Based Matching (PBM) algorithym. Several manufacturers offer a form of PBM that is limited to a comparison by a forward search of the spectra of single pure (unknown) compounds with spectra stored in McLafferty's very large library of mass spectra at Cornell University. However, since we are dealing with mass spectral patterns generated from human body vapors, which are composed of hundreds, if not thousands, of organic compounds in exceedingly complex mixtures, it is obvious that it is necessary to generate a novel kind

DISCUSSION (continued)

of library that contains the spectra of unique mixtures, rather than single compounds. Greatly enhanced specificity is obtainable if the unknown spectrum is examined for the presence of required characteristics of the reference spectrum, (this process is known as "reverse search").

The only mass spectrometer system which allows the comparison by reverse search PBM of an unknown spectrum, with reference spectra that have been generated and stored in a library residing in the instrument itself, was designed and produced by Universal Monitor Corporation (UMC) of Pasadena, California, in collaboration with Professor McLafferty. Unfortunately, Universal Monitor is no longer in business. However, the consultant on this project, Dr. Green, kindly loaned us a prototype instrument which he had obtained from UMC more than ten years ago.

While this instrument system, which UMC called Olfax I, contained the necessary mass spectrometer, air sampling inlet and a computer having PBM software, it was very old, having been fabricated in the late 1960's and stored in a barn in a disassembled state for four years. By the application of considerable effort and ingenuity, functions work relatively well. However, because of its mechanical relays and old wiring, which caused many shorts and ground loop problems, the spectrometer was sometimes unstable and unreliable. This level of performance did not provide precisely reproducible spectra and, therefore, did not allow the critical comparison of unknown and reference spectra that this project required.

COLLECTION CHAMBERS AND TRAPS

The whole body Collection Chamber, fabricated at our facilities for use in our experiments, measured 24" x 24" x 72" (h) and was constructed of a wooden frame which was lapped together and fitted with a hinged air tight door. The Chamber interior and door were lined with five (5) mil Kapton plastic film, which was tightly sealed by pressing round, rubber cord into grooves running along the chamber's outside edges for an airtight enclosure.

An activated charcoal filter, $3\frac{1}{2}$" (dia) x 14" (l), attached to the lower portion of the chamber, was connected to a low volume oil free pump and a sample collecting cartridge (trap) was installed at the top of the chamber. A stainless steel valve was fitted at each end of the trap in order to retain vapor sample.

To ensure transfer of effluents from the chamber and sample trap to the Olfax instrument, the effluent line was heated at various temperatures until it was determined an optimum temperature. The effluent line, constructed of 1/16" (dia) 304 stainless steel tubing, was enclosed in an insulated heat chamber and temperature controlled ±1C over its length.

COLLECTION CHAMBERS AND TRAPS (continued)

In preparation for sample collection, each trap was cleaned by simultaneously pumping nitrogen through the trap while heating to 275° for 8 to 12 hours. The trap was disconnected from the pumping and heating system, cooled to room temperature, reattached to the collection chamber (through which fresh air was being pumped), and an air sample was collected by applying vacuum to the trap. The trap was removed from the chamber, heated at 150°C to desorb any accumulated vapors and a background scan was run to determine if any compounds were present. Prior to a subject entering the chamber, the interior was purged for at least two hours with air that had been cleaned by passing it through an activated charcoal trap. After a subject entered through a door made up of one side of the chamber, he stood in the center on a clean paper towel for thirty to ninety minutes, being careful not to touch the sides of the chamber. Clean air from the charcoal filter attached to the lower portion of the chamber and connected to a low volume were withdrawn through a tube at the chamber top through traps that collected the organic vapors.

The sample collection trap, which was fitted with a stainless steel valve on each end, in order to isolate the sample when desired, and to control air flow, i.e., elution rate, was again attached to the top of the chamber for sample collection. Vapors were pulled through the trap with a 1.1 cu/ft per minute, oil free pump which was connected to the bottom of the sample trap (see figure 1). The trap was detached after specimen collection was completed and attached directly to the analyzer inlet.

As expected, when either the charcoal or 13X molecular sieve trap was connected to the chamber for sampling, the quantity of water which collected, along with the organic vapors, was more than the pumping system of the spectrometer could tolerate. Thereafter, a 3X molecular sieve trap was attached directly to the chamber to remove most of the water as it left the chamber so that only a minimum of moisture passed into the sampling trap, along with the desired vapors.

The first three types of sample traps evaluated were 2"x8" aluminum cannisters filled with 1) activated charcoal, 2) 3X molecular sieve, or 3) 13X molecular sieve. The traps, as well as the inlet and analyzer, were assayed over temperature ranges from 225°C as a maximum, to room temperature (approximately 81°F) as a minimum. We confirmed that; the most favorable sample collection occurs at room temperature; compounds with high molecular weights require high inlet and analyzer temps when samples are introduced into the system in order to avoid "plating out." The optimum temperature and flow rates for analysis and sample collection were as follows:

	TEMPERATURES	FLOW RATE
Analyzer	150°C	600cc per min
Analyzer Inlet	125°C to 150°C	600cc per min
Membrane Separator	125°C to 180°C	600cc per min
13X Sieve trap	150°C	530 to 600cc per min
Charcoal trap	200°C	520 to 580cc per min

COLLECTION CHAMBERS AND TRAPS (continued)

In addition, analyzer pressures of 1.5×10^{-6} to 2.0×10^{-6} torr, provided the most useful data.

After vapor collection, the traps were desorbed by heating them (without flow) for 15 minutes at 150°C by application of a heating tape wound around the aluminum cannister. The trap was then attached to the analyzer, and eluted with a flow of dry nitrogen to introduce the specimen into the analyzer. Our evaluation disclosed these traps to be unsatisfactory because they contained so much adsorbant that the desorbed samples seriously overloaded the analyzer, causing a persistent elevation of the background. Moreover, their size precluded heating and elution of samples.

In an attempt to obtain more suitable traps; 1/2" O.D. x 6" long stainless steel tubes were filled with 13X molecular sieve material. These traps were more satisfactory than the 2" cylinders, but still had more capacity than desired. New traps, which we fashioned from 1/4" O.D. x 6", glass lines stainless steel tubing, proved to be best for our experiments. At this time, we also changed from 13X molecular sieve to Tenax TA adsorbant. This combination of smaller traps and Tenax TA enabled us to control the sample introduction rate with sufficient percision. These traps were used in the majority of the succeeding tests.

MINI-CHAMBER

In order to obviate the need for the subject to stand motionless in the body chamber for 30 minute periods or longer, and to reduce the time spent in decontaminating and checking the 600 liter clean chamber, a much smaller and more simple "chamber" was fashioned from a two pound coffee can. For sampling, a subject's soiled undershirt was placed in a clean 2.0 liter can. The can was connected via a length of tight fitting teflon tubing inserted through the plastic container lid leading to a Tenax TA trap. The can remained at room temperature of approximately 38"C, clean air was introduced and the vapors which emanated were conducted into the trap for thirty minutes. The trap was then connected to a heater with temperature controller and held at 150°C for 15 minutes. Attaching the trap to the inlet, with the temperature remaining constant, and eluting the sample with a flow of nitrogen measuring between 550cc to 600cc per minute gave optimum results.

The use of these mini-chambers allowed the collection of larger samples in shorter times. When spectra of a subjects' garments in a 2.0 liter "chamber" were compared with spectra of that subject in the 600 liter whole body chamber, there was little apparent difference.

In later tests the Tenax TA traps were eliminated from the system and the mini-chamber was then heated to 50°C and attached directly to the analyzer inlet. High sample yields were obtained with essentially unchanged spectra when using this technique for analysis.

SKIN OIL STUDIES

In order to resolve the problems associated with trapping vapor samples using the large 2" x 8" traps, and to diminish sample collection time, we developed and evaluated techniques for introducing skin oil samples by direct injection and by external vaporization. For direct injection a syringe needle was fitted with a protective sheath. After thoroughly cleaning the needle and sheath, sample was collected by pressing the needle along side the nose and rotating it to coat it with oil. The sheath was then placed over the needle and the assembly was inserted through a rubber septum into the heated mass spectrometer inlet for analysis. The sheath was withdrawn, allowing the sample to vaporize from the needle and to enter the analyzer through the silicone rubber molecular separator.

As an alternative introduction method, skin oil was collected by rubbing a clean unlighted 250 watt, high intensity halogen lamp on the side of the subject's nose. The lamp was held below a funnel through which air was drawn into the inlet. The lamp was then set at 175 watts producing a surface temperature of 500°C. This rapidly vaporized the oil which coated the surface of the bulb. As with the direct injection technique, samples were more than adequate, but sample sizes were difficult to control, and occassional analyzer overloads required long times for recovery. Both of these methods produced very similar mass spectra.

CHEMICAL SIGNATURE

Although the spectrometer did not behave well enough to produce really definitive data on the differences between the mass spectra of our subjects, it did work well enough to indicate that there were differences and that compounds were abundant enough to give signals that were in many cases one hundred to three hundred times background.

Most of our measurements were confined to the mass range m/z 45 to 205. As was expected, we observed clusters of ions with maxima at about m/z 55 and 57; 69 and 71; 81 and 83; 95 and 97 and so on, i.e., about every 14 amu. Their intensities decreased approximately, logarithmically at about one decade per 50 amu. As an indication of the types of data that were obtained during those periods when our instrument appeared to be functioning properly, Figure 2 shows the ratios of the ion currents (corrected for background) at individual m/z's in the spectra of two subjects (one female and one male). Thus, when the currents were identical, a ratio of 1.0 was obtained. When an arbitrarily assigned range of +20% is applied to the data, 52 of the 97 usable nominal masses between m/z 50 and m/z 150 were found to differ from the corresponding ion in the paired spectrum by more than 20%. Such differences are easily detected by reverse search PBM, so it does appear that the premise, upon which our proposal was based, is valid. Confirmation can easily be obtained by repeating such tests using a reliable instrument. In addition, a detection limit in the low parts per billion range was observed.

RECOMMENDATIONS

In the Phase I, SBIR research study, Environmental Devices, Incorporated, demonstrated the feasibility of a human chemical identifier by showing that there are differences in the effluents emmitted by individual subjects. Our laboratory experiments revealed that reproducible data can be obtained from individual subjects which distinguishes one person from another by the variations of chemical signals of the subjects.

In order to continue this research, it is recommended that the Principal Investigator have use of a mass spectrometer with high resolution and greater stability for future experiments in order to isolate and identify discrete chemical compounds found during the Phase I experiments. The benefits of having both the late model Olfax and a modern spectrometer will allow the vital comparison and accuracy of data, and will aid in reducing reporting time and repeat experiments. In addition, the two instruments will assist in the testing and development of a protomodel instrument.

It is recommended that Environmental Devices be funded for the second phase of the project to enable staff to complete the latter stages of research and to begin the design and development of an instrument.

FUNDING/EXPENDITURES

In our Phase I proposal, we forecast expenditures of $77,871.70 for the six month period. Upon notification of approval of the award, we were advised that the proposal must be cut 36% due to SBIR funding limitations. Careful consideration was given to the costs the corporation might incur in order to fulfill the contract. It was decided that by eliminating equipment leasing, decreasing consultation time and travel, and by keeping a close watch on all expenses associated with the project, we could cut the corporation's share of the funds to a minimum and still take advantage of an important research opportunity.

Total project expenditures of $49,302.05, which does not include unclaimed expenses during March for consultant fees, travel, overhead costs and some salary, translates to the following percentages:

Wages and labor	67%
Overhead	15%
Consultant fees	8%
Direct material costs	7%
Travel, lodging, meals	3%

In tallying contractor costs for the six month project, expenses ran five percent (5%) above the total grant allocation, a small price for us to pay for a valuable research opportunity.

In retrospect, our decisions to eliminate the leasing of a more modern mass spectrometer system and to reduce consultant time and travel, very adversely affected our ability to deliver the quality of results anticipated in our initial proposal.

Since completing Phase I, we have acquired the latest spectrometer of its kind, the Olfax IIA. With some refinements and additions we are continuing research on this project with the intention of applying for the Phase II effort.

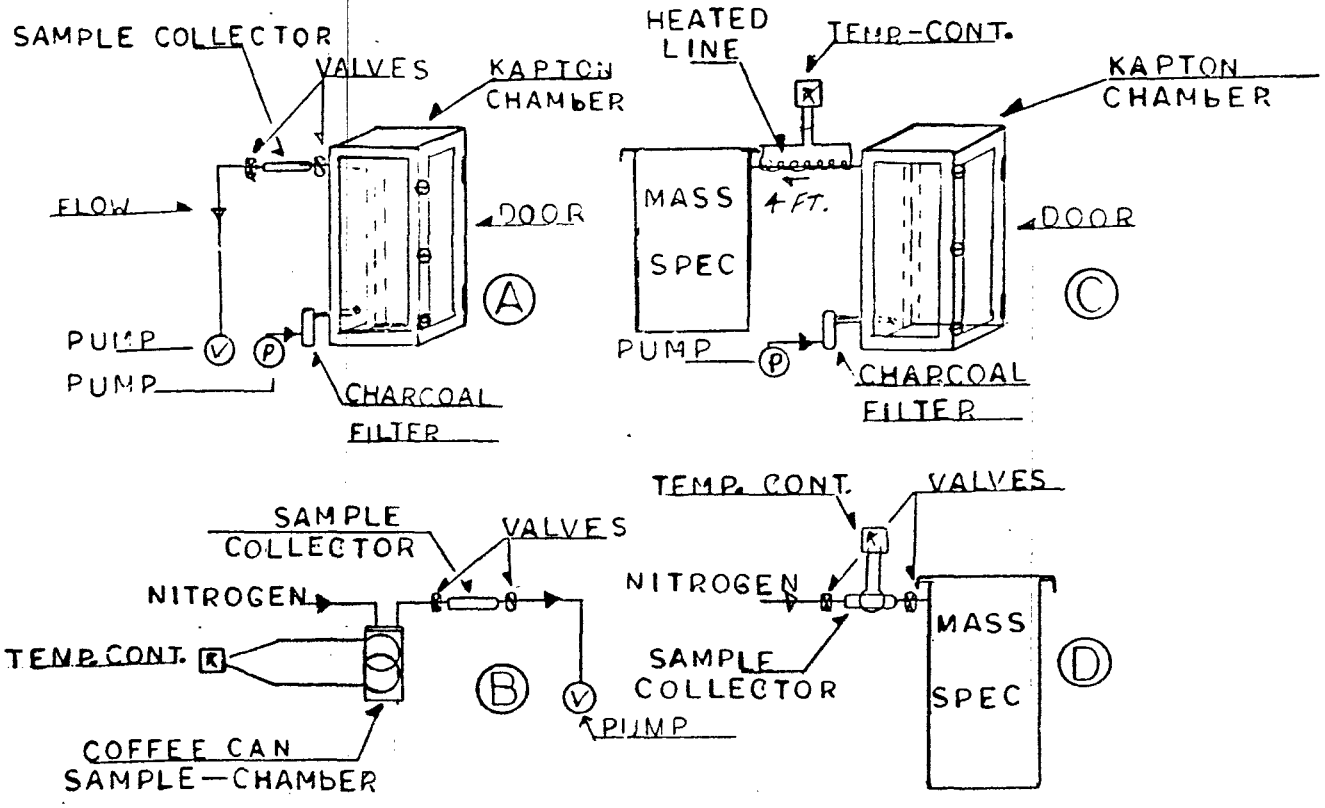
FOOTNOTES

1) AN APPARATUS FOR THE DETECTION AND QUANTITATION OF VOLATILE HUMAN EFFLUENTS, R.I. Ellin, et al, Journal of Chromotography, 100 (1974) 137-152, Elsevier Scientific Publishing Co., Amsterdam.

2) A STUDY ON THE IDENTIFICATION AND DETECTION OF THE VOLATILES OF CONTRABAND FOODSTUFFS, U.S. Dept. of Agriculture (APHIS), 1981.

3) PBM, PROBABILITY BASED MATCHING, an algorithmic system developed and copyrighted by Fred W. McLafferty, Ph.D.

4) ALGORITHMS, Robert Sedgewich, Addison-Wesley Publishing Company, Reading, Massachusetts, 1983.



SAMPLE COLLECTION AND INTRODUCTION TECHNIQUES

FIGURE 1

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APPENDIX A

Data are generated and presented by the Olfax system in an unconventional manner. The attached spectra, which are normalized to the highest ion current, illustrate some of the unique features of the Olfax software.

All data are digital. Ion currents are reported in amperes. (The maximum signal in the spectrum of Subject B was 7.7 x 10-7 amps, and the one for the spectrum of Subject N was 2.1 x 10-6 amps). Relative abundances in spectra of pure compounds have been found to be reproducible even when ions are less than 0.01% of the base peak. Therefore, it is common to display spectra in a three or four-decade logarithmic format. (Because of the manner in which data can be recorded, it is even valid to display some spectra over six-decades).[4]

The attached spectra were used to derive the data in Figure 2 of our final report by taking the ratio of the signals in Spectrum N to the corresponding signals in spectrum B. (Since these are logarithmic presentations, this can be achieved by merely subtracting the height of B from the height of N, mass for mass). Unfortunately, when these experiments were conducted, we failed to print the fourth decade, so the data for the smallest ions was not recorded, i.e., m/z 58 and 59. Where data are recorded in one spectrum but not in the other, i.e., m/z 75, only a limiting value can be calculated.

In the program which was employed to generate these particular sets of data, an error in the software causes an unexplained "glitch" in multiples of 32 amu, i.e., @ m/z 64, 96, 128.... Therefore, the ion currents at those masses were not used in the computations. (It is possible to measure these ion currents accurately by using a different type of program in the Olfax system, when desired).

The sensitivity of the method we used as our example is more than adequate as evidenced by the ion currents displayed. In one case, the baseline is at 7.7 x 10-10 amps. This is about ten (10) times greater than the instrument background.

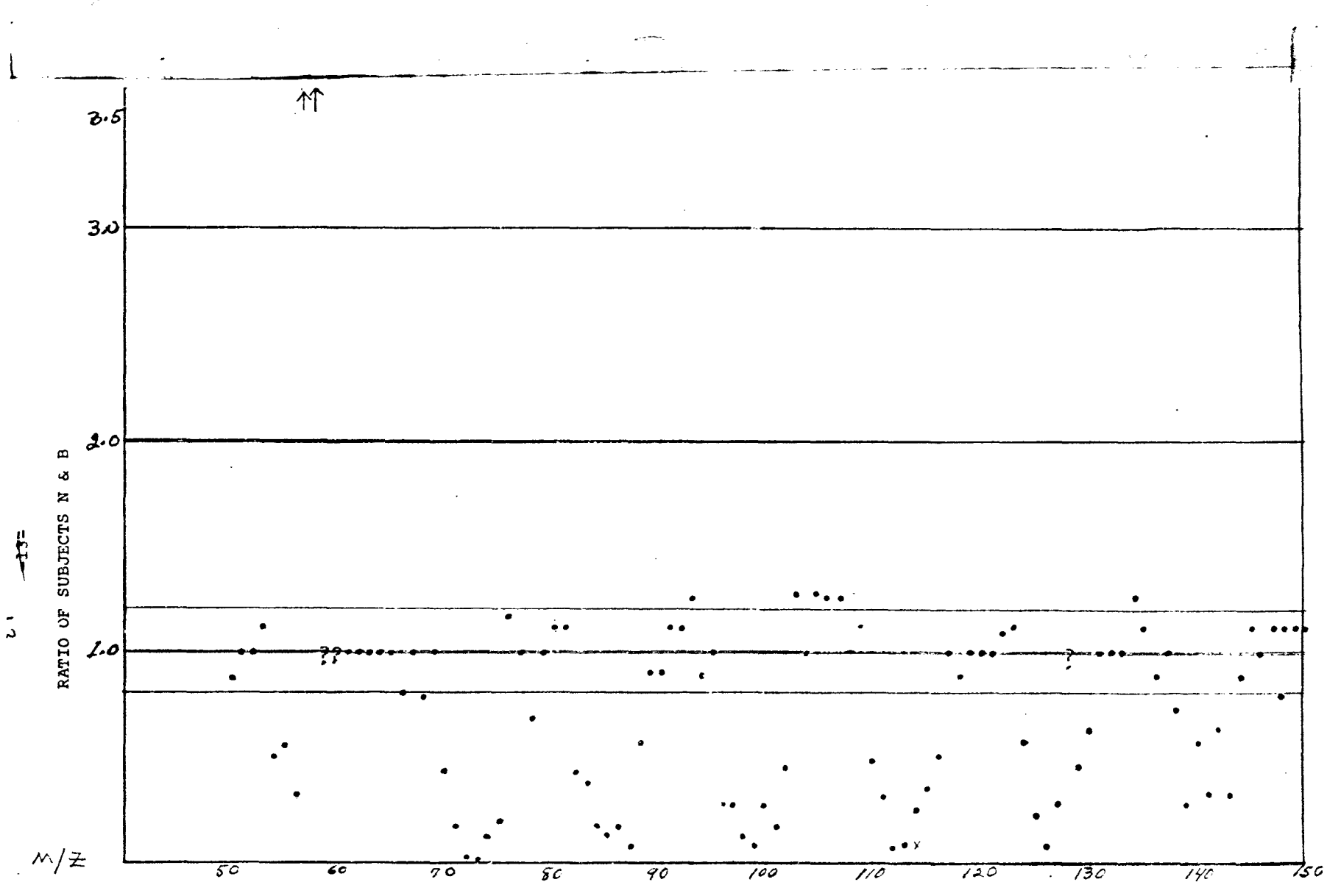
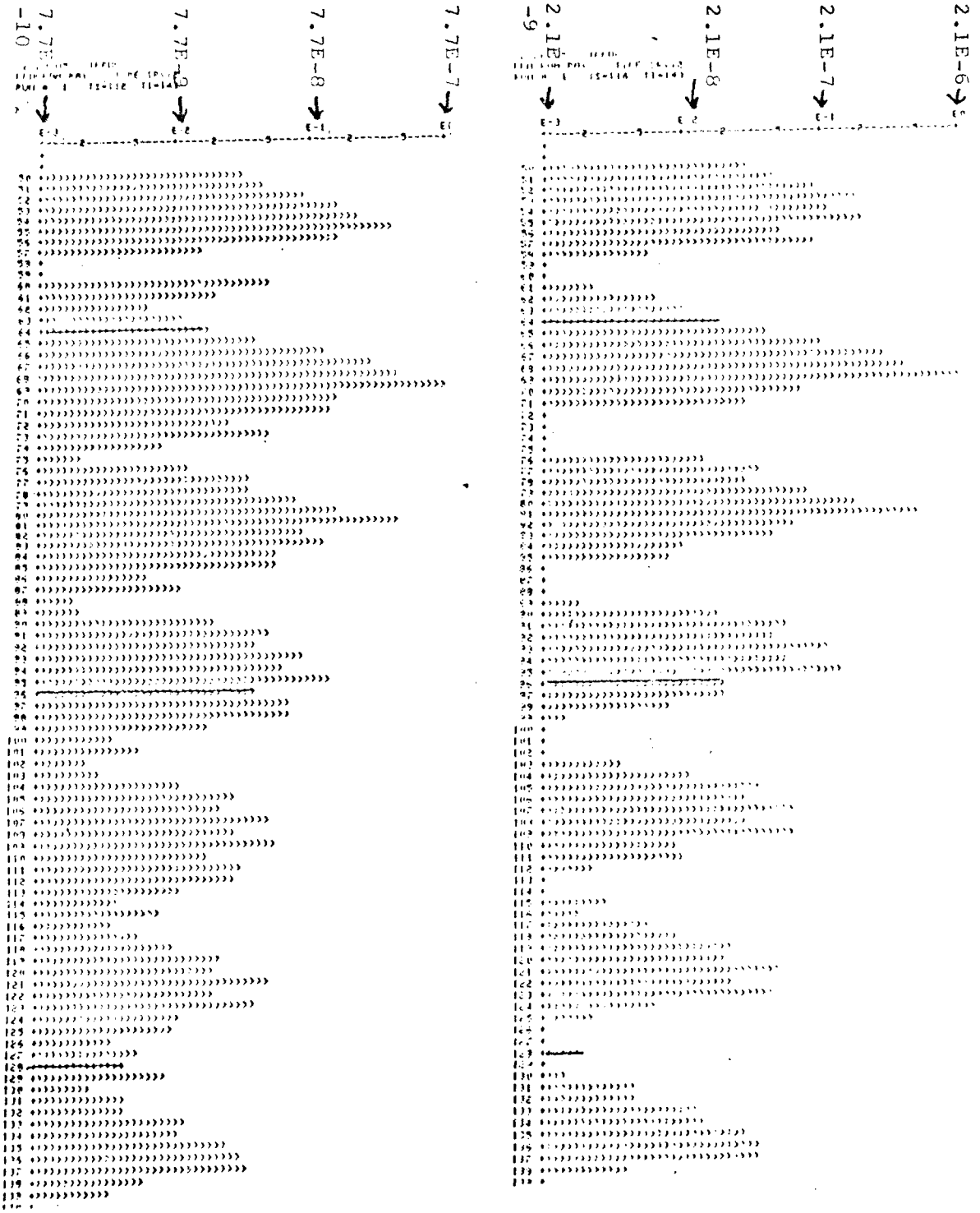

3.5
3.0
2.0
1.0
RATIO OF SUBJECTS N & B
M/Z
50
60
70
80
90
100
110
120
130
140
150

FIG. 2



Partial spectra of SUBJECT B SUBJECT N

(Displayed in a three-decade logarithmic format, after subtraction of background)

SPECTRUM RATIOS SUBJECTS N & B

M/Z		M/Z	
50	0.88	104	1.00
51	1.00	105	1.29
52	1.00	106	1.27
53	1.13	107	1.26
54	0.50	108	1.00
55	0.55	109	1.13
56	0.32	110	0.49
57	5.56	111	0.31
58	>5.56	112	0.08
59	?	113	≤0.09
60	?	114	≤0.25
61	1.00	115	0.35
62	1.00	116	0.50
63	1.00	117	1.00
64	1.00	118	0.89
65	1.00	119	1.00
66	0.80	120	1.00
67	1.00	121	1.00
68	0.79	122	1.10
69	1.00	123	1.13
70	0.44	124	0.57
71	0.18	125	0.22
72	≤0.04	126	≤0.09
73	≤0.02	127	≤0.28
74	≤0.13	128	?
75	≤0.20	129	≤0.45
76	1.18	130	0.63
77	1.00	131	1.00
78	0.78	132	1.00
79	1.00	133	1.00
80	1.12	134	1.26
81	1.12	135	1.12
82	0.44	136	0.89
83	0.36	137	1.00
84	0.18	138	0.71
85	0.14	139	≤0.28
86	<0.16	140	≤0.56
87	<0.09	141	0.32
88	<0.56	142	0.63
89	0.90	143	≤0.32
90	0.90	144	0.89
91	1.12	145	1.12
92	1.11	146	1.00
93	1.26	147	1.12
94	0.89	148	0.79
95	1.00	149	1.12
96		150	1.12
97	0.28		
98	0.12		
99	0.08		
100	<0.28		
101	<0.18		
102	<0.45		
103	1.29		

COINCIDENTIAL CHEMICAL SIGNATURE IDENTIFIER

MILESTONE CHART

PHASE I